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Supplementary appendix

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Supplementary Appendix

The Natural History of Alzheimer´s Disease in adults with Down Syndrome: a cross-sectional study

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Methods

Reference Population

This study was performed at two centers, Barcelona (Spain) and Cambridge (United Kingdom). The cohort of adults with Down syndrome in Barcelona was recruited from a population-based health plan. The Table S1 compares the demographics and clinical diagnoses of the adults with Down syndrome included in the study and those that were not included but who were evaluated in the health plan. The total number of subjects with DS that were included in the Barcelona health plan during the study period (Feb 1, 2013 - Jul 31, 2019) was 659, of whom 347 entered the study. There were no differences in the demographics, although there were differences in the degree of intellectual disability and in clinical diagnosis categories between the two subsamples. Those included were more likely to have milder levels of intellectual disability and have symptomatic Alzheimer´s disease.

	Not included	Included	*p*-value
N	298	347	
Male, N (%)	158 (53·0%)	188 (54·2%)	NS
Female, N (%)	140 (47·0%)	159 (45·8%)	
Age (median [IQR])	43·6 [18·8]	45·2 [16·4]	NS
Intellectual disability, N (%)			p=0·0004
Mild	45 (15·1%)	72 (20·7%)	
Moderate	148 (49·7%)	177 (51·0%)	
Severe	66 (22·1%)	79 (22·8%)	
Profound	39 (13·1%)	19 (5·5%)	
Diagnosis, N (%)			p=0·01083
aDS	224 (75·2%)	224 (64·6%)	
pDS	18 (6·0%)	36 (10·4%)	
dDS	56 (18·8%)	87 (25·1%)	

Table S1. Included and not included subjects in the study for the Sant Pau center. Abbreviations: aDS: asymptomatic Down syndrome group; dDS: Alzheimer disease dementia in Down syndrome group; IQR: interquartilic range; pDS: prodromal Alzheimer disease in Down syndrome group.

The cohort of adults with Down syndrome in Cambridge and the euploid controls were convenience samples. As mentioned in the main text, Down syndrome participants from the Cambridge cohort were recruited via services for people with intellectual disabilities in England and Scotland and with the support of the UK Down Syndrome Association.

We also selected a convenience sample of non-trisomic controls from the Sant Pau Initiative on Neurodegeneration (age≤75).[1] They were recruited from the general population in the metropolitan area of Barcelona during the same period and using the same procedures as the Down Alzheimer participants. Most euploid controls were recruited among carers and family members of patients attending the memory unit.

Structural imaging

MRI acquisition

In this study we included data from two centers: Hospital de Sant Pau Barcelona, Spain and Cambridge, United Kingdom. Of the 337 MRIs included, 297 (81·1%) were from the Hospital de Sant Pau and, the remaining 40 (11·9%) were from Cambridge. All Cambridge participants are subjects with Down syndrome. Acquisitions parameters for both centers are explained in the Table S2.

	Hospital de Sant Pau	Cambridge
Scanner	PHILIPS 3.T X SERIES ACHIEVA	Siemens Verio 3T
Protocol	MPRAGE	MPRAGE
Repetition Time (ms)	8·1	2300
Echo Time (ms)	3·7	2·98
Slices	160	176
Voxel Size (mm)	0·94x0·94x0·94	1x1x1

Table S2: MRI acquisition parameters for structural MRI for Hospital de Sant Pau and Cambridge

MRI processing

All images were visually read by an expert radiologist to check for major abnormalities. Then, structural images were processed with Freesurfer software package (version 6, http://surfer.nmr.mgh.harvard.edu) to obtain each subject cortical reconstruction, using a procedure described in detail elsewhere.[2] All reconstructions were visually inspected in a slice-by-slice basis by an experimented engineer and corrected when necessary. In this process, 36 images (32 from participants with Down syndrome and 4 from euploid controls) were discarded due to movement artifacts (28), poor contrast (4) or major segmentation errors (4). Once the images were processed, we extracted the hippocampal volumes and we computed the adjusted hippocampal volumes as previously described.[3] Finally, for the surface-based analyses, cortical reconstructions were smoothed with a 15 mm FWHM gaussian filter.

FDG-PET

FDG acquisition

A subset of participants (n=197, 31·3%) underwent a 18F-FDG PET acquisition at the Sant Pau Hospital, Barcelona. The acquisition was performed in two different scanners:

GEMINI

Fasting patients with a glycemia < 140 mg/dl were injected an intravenous dose of 259Mbq (7mCi) of 18F-FDG. The patient rested 60 minutes in an isolated and dark room. Images were then acquired with a scanner Gemini TF PET-TC. The acquisition protocol consisted of a topogram, TC (mA 100 and Kv=30), and 3D PET with TOF. The parameters of the reconstructed image with the BrainSmooth filter were: FOV 250mm, 3mm slice thickness in a 512x512 matrix.

VEREOS

FDG-PET was acquired according to the Sant Pau Hospital protocol. Briefly, fasting patients with a glycemia < 140 mg/dl were scanned with a Vereos PET/TC Phillips scanner 60 minutes after a 259Mbq (7mCi) injection of intravenous 18F-FDG. The acquisition protocol consisted of a topogram, TC (mA 100 and Kv=30), and 3D PET with TOF. The parameters of the reconstructed image with the BrainSmooth filter were: FOV 256mm, 164 slices in a 256x256 matrix with a voxel dimension of 1x1x1mm.

FDG processing and SUVR extraction

All images were visually inspected by expert nuclear medicine specialists before any processing to check for brain abnormalities. Then, FDG-PET images were entered to the Sant Pau FDG-PET processing stream.[1] The quantitative analysis was performed as follows: first, PET images were spatially normalized to the MNI space using the SPM12 https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) PET template. Then, the normalized images were scaled by the pons-vermis region and finally a Standardized Uptake Value Ratio (SUVR) was calculated using the MetaROI described by Landau and colleagues in 2011[4] (Left Angular Gyrus, Right Angular Gyrus, BilateralPosterior Cingular, Left Inferior Temporal Gyrus, Right Inferior Temporal Gyrus).

FDG surface analysis

Additionally, 70% of subjects had an FDG-PET had an MRI available with good quality that can be used to conduct surface-based group analysis (n=71). Basically, FDG-PET images were normalized by the reference region, coregistered to each individual structural image and PET values were projected to the cortical surface. Finally, individual surfaces were smoothed with a 15mm FWHM gaussian kernel and introduced to the analyses.

Amyloid PET

FBP acquisition

Florbetapir-PET was acquired in Barcelona according to the Sant Pau Hospital protocol. Briefly, patient was scanned with a PHILIPS GEMINI TF PET-TC after a 370 Mbq (10mCi) of 18F-Florbetapir followed

by physiological serum. The acquisition protocol consisted of a topogram, TC (mA 100 and Kv=30), and 3D PET with TOF. The reconstruction used a Lor Ramla filter, FOV 256mm, voxel dimension of 2x2x2mm and 89 slices in a 128x128 matrix.

PIB acquisition

PIB- PET was acquired in Cambridge participants following previously published methods.[5] Briefly, patients were scanned in a three-dimensional mode on a GE Advance scanner after an injection of a bolus of 11C-PIB (545 MBq). Data were acquired for 90 minutes after injection in 58 frames (18x5 seconds, 6x15 seconds, 10x30 seconds, 7x1 minute, 4x2·5 minutes, and 13x5 minutes. For each frame, sinogram data were reconstructed using the PROMIS 3D filtered back projection algorithm into a 128x128x35 image array with a voxel size of 2·34x2·34x4·25 mm3. Corrections were applied for random coincidences, dead time, normalization, scatter, attenuation, and sensitivity. Finally, for the purpose of this study, frames from the period 50-70 minutes were selected, realigned and averaged to obtain a single mean image.

Centiloid conversion

Two different amyloid tracers were used in this work. We used the centiloid scale to unify binding SUVRs using standard procedures.[6] First, we downloaded the sample PIB dataset from GAAIN (http://www.gaain.org/centiloid-project) and we computed the mean SUVRs. Briefly, PET-PIB images were coregistered to their corresponding MRI and then normalized to the MNI space. Mean binding SUVR from predetermined VOIs (volume of interest) were computed. The SUVR was defined as the cortical to whole cerebellum VOIs ratio, as provided by the authors. Once this step was accomplished, we performed the calibration step. I.e., we validated our PIB image registration pipeline and we verified that the in-house obtained centiloid values matched the standard data provided and falls within the expected margins (Figure S1).

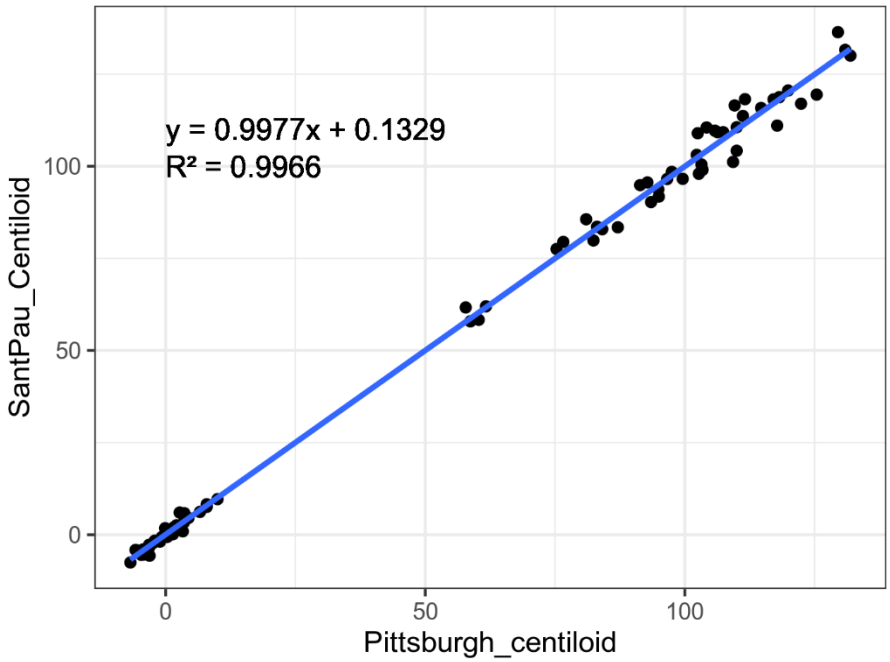

Figure S1. Correlation of the Sant pau and Pittsburgh analysis using the whole cerebellum volume of interest as recommended in Klunk 2015.

Klunk and colleagues recommended that for this calibration step, the slope of the regression should fall within the range [0·98, 1·02], the intercept within the range [-2, 2] and the R^2>0·98. All three conditions are met in our analysis. Then, following standard procedures we conducted the Level-2 analysis: we computed the PIB-centiloid regression using the in-house calculated young controls and Alzheimer's disease SUVRs. In this case, the mean should fall within 2% of the reported means in Klunk 2015. The mean values for our data were 2·08 for AD subjects and 1·01 for the young controls, both falling within the specified range. Finally, the following formula was applied to convert PIB to centiloid values:

Centiloid=100* (PIB-1·01)/(2·08-1·01)

Equation 1. Formula to convert PIB SUVR to the centiloid scale

Then, to convert the Florbetapir images to the centiloid scale we downloaded the Florbetapir calibration dataset from GAAIN,[7] computed the mean Florbetapir and PIB SUVR using exactly the same procedure described above, and we computed the PIB – Florbetapir regression (Figure S2).

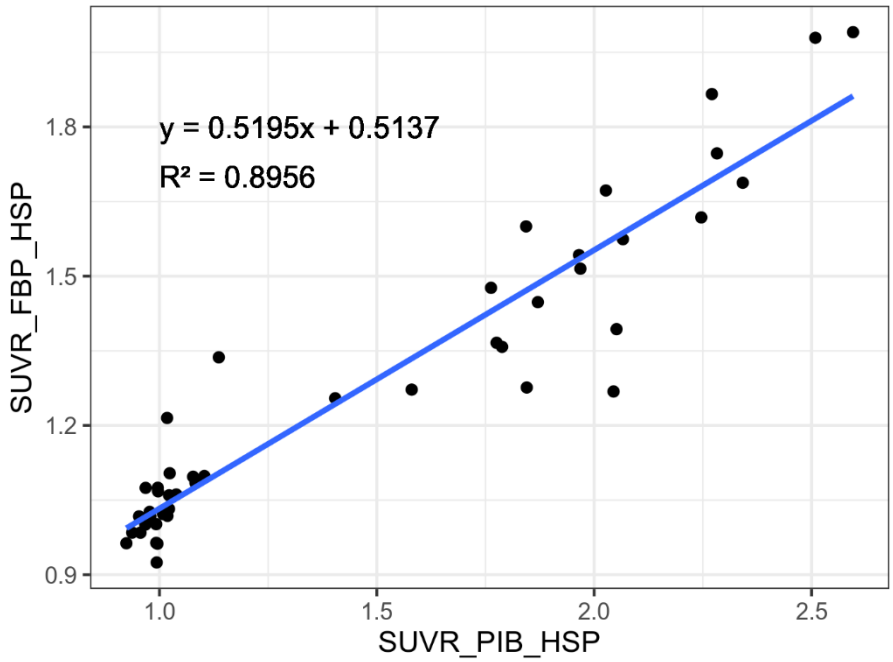

Figure S2. Correlation between Florbetapir and PIB SUVRs using the publicly available dataset from Navitsky and collleagues.

This regression, in conjunction with the Equation 1, was used to convert the Florbetapir SUVR to the Centiloid scale. Finally, each subject had a Centiloid quantification. The comparison of the tracer retention evolution with age between centers (and tracers [Florbetapir and PIB]) expressed in centiloid units can be found in the Figure S3.

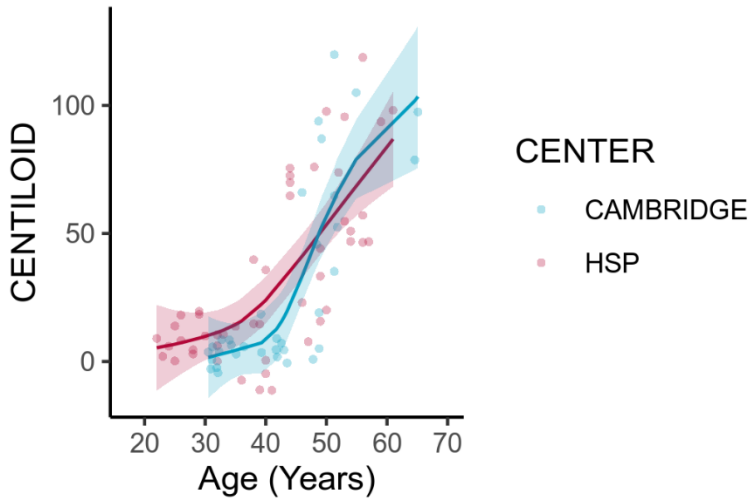

Figure S3. Comparison of the age evolution between centers (Cambridge and Hospital de Sant Pau) and tracers (PIB and Florbetapir). HSP=Hospital de Sant Pau.

Florbetapir on surface

For those subjects that had Florbetapir-PET and MRI available (n=42) we conducted surface-based group analyses. Basically, Florbetapir-PET images were normalized by the reference region (whole cerebellum), coregistered to each individual structural image and PET values were projected to the cortical surface. Finally, individual surfaces were smoothed with a 15mm FWHM gaussian kernel and introduced to the analyses.

Biochemical analysis

Cerebrospinal fluid analysis

All CSF analyses were conducted at the Hospital de Sant Pau using a procedure described elsewhere,[1] following international recommendations. After lumbar puncture, the first 2 ml of CSF were transferred to the general laboratory for routine testing. Another volume of 15-20 ml was transferred to the Memory Unit laboratory where samples are processed and aliquoted within the first two hours after lumbar puncture. CSF samples were stored at -80ºC until analysis. CSF levels of Aß42, Aß40, phosphorylated tau and total tau were measured using the automated platform Lumipulse G600 (Fujirebio-Europe), using the first freeze-thaw cycle for each sample. Neurofilament light chain levels were measured with a commercially available ELISA (UmanDiagnostics, Umeå, Sweden) following manufacturer's recommendations.

Plasma analysis

Plasma samples were collected in 10ml EDTA tubes, and subsequently processed, aliquoted and stored at -80ºC as previously described according to standardized procedures.[8] Plasma samples were analyzed at Centre Hospitalier Universitaire Montpellier (n=411, Montpellier, France) and at Hospital de Sant Pau (n=17, Barcelona, Spain) using the ultrasensitive Single Molecule Array (SIMOA) technology with commercially available kits (Neurology 3-Plex A for AB40, AB42 and Tau; NF-light Simoa Assay Advantage Kit for NfL). The analyses were run in the SIMOA HD-1 equipment (Montpellier) and in the SIMOA SR-X equipment (Barcelona). To ensure compatibility between centers we performed repeated measurements of 10 individuals in both centers.

Samples were analyzed in simplex or duplicates (about 15% of total analyzed samples in Montpellier and all samples analyzed in Barcelona). All samples were 4-fold diluted online with the provided dilution buffer (phosphate buffer, containing bovine serum and heterophilic blocker solution) to minimize matrix effects.

Quality controls provided in the kits with low and high known concentration of NfL, t-tTau, Aβ1-40, or Aβ1-42 and one internal QC corresponding to pooled plasma were analyzed in every plate to assess assay performance. Details on the assay performance are provided in Table S3.

	Aβ1-42	Aβ1-40	t-tau	NfL
Mean intra-assay CV% (Montpellier)	6%	4%	13%	6%
Mean inter-assay CV% (Montpellier)	12%	7%	5%	10%
Mean intra-assay CV% (Barcelona)	0·5%	2·2%	2·4%	6·7%
Mean inter-assay CV% (Barcelona)	9·5%	4·2%	17·2%	4%
Mean inter-center CV%	12·6%	13·7%	29·7%	11·7%

Table S3. Details on the assay performance

Statistical analyses

Power calculation and missing handling

This study included all data available in each biomarker modality in order to maximize the statistical power. Thus, subjects who did not have data for a concrete biomarker were simply not included for that biomarker only, but were included for the others. No imputation procedures were applied.

Software used

Statistical analyses were performed with in-house developed scripts fully written in R (Version 3.6.1).

Baseline differences

Baseline differences between groups were assessed with Kruskal-Wallis tests and pairwise Wilcoxon test corrected for multiple comparison ($p<0{\cdot}05$). Differences in group proportion (sex, and cognitive disability level) were tested with chi-square tests ($p<0{\cdot}05$).

Imaging analyses

Brain image analyses were performed with Freesurfer tools. For structural MRI, FDG- and amyloid-PET brain surfaces were entered into group analyses with diagnosis as group (symptomatic vs asymptomatic down syndrome patients) and gender as covariate. To avoid false positives, we run a Monte Carlo simulation with 10000 repeats as implemented in Freesurfer (Family Wise Error [FWE] correction). Thus, only results that survive an FWE $p<0{\cdot}01$ were displayed. Results for atrophy and hypometabolism are displayed in blue/light blue tones to denote a reduction in symptomatic patients compared to the asymptomatic ones, whereas amyloid results are displayed in red/yellow tones to denote the inverse effect.

Temporality of biomarker changes

To assess the temporality of change for each biomarker we fitted 1st degree Loess curves with 95% confidence intervals for the group of participants with Down syndrome and the general population normal controls, separately (Figure 3), as in Bateman and collaborators.[9] The age at which the confidence intervals first do not overlap is considered the age of biomarker change. When the fitted curves by group clearly differed from young ages (i.e, plasma Aß42/40 or HVa) the curves were visually described. Figure 4 was generated with the same final models and the standardized difference is plotted. In other words, to compute the predicted difference we subtracted the control curve to the Down syndrome curve, and we divided the result by the standard deviation of the corresponding biomarker.

Results

As explained in the main text, sample sizes for the different biomarker modalities are different. More details can be found in the Table S4. When comparing the subsamples of participants with Down syndrome, there were no differences in age, gender distribution or disability level between the subsamples. There were differences, however, in the diagnostic distribution. The CSF and FDG samples had a higher proportion of symptomatic subjects. In the control group, the CSF and MRI samples were older compared to the plasma sample (Kruskal wallis p<0·001, Wilcoxon paired test p<0·05). No differences were found in gender distribution in the control group.

Biomarker		samples	Age (median [IQR])	Female (%)	Diagnosis split in participants with DS aDS/pDS/dDS N [%]	ID split in participants with DS (Mild/Moderate/Severe or profound) N [%]
CSF	Down	137	47·7[14·0]	45·5	73-26-38 [53·3-19·0-27·7]	30-77-30 [% 21·9-56·2-21·9]
	HC	207	57·1 [11·7]	65·0		
Plasma	Down	323	44·5 [16·6]	46·1	214-37-72 [66·2-11·5-22·3]	65-167-91 [20·1-51·7-28·1]
	HC	112	53·1 [11·9]	68·8		
Centiloid	Down	83	41·8 [16·9]	36·6	57-15-11 [68·7-18·1-13·3]	13-29-7 [26·5-59·2-14·3]
	HC	25	56·1 [10·0]	64·0		
FDG SUVR	Down	107	47·2 [14·6]	50·0	59-12-36 [55·1-11·2-33·6]	26-56-25 [24·5-51·9-23·6]
	HC	90	56·0 [14·9]	68·9		
Hva	Down	170	43·6 [16·4]	40·1	115-27-28 [67·6-15·9-16·5]	36-75-20 [27·5-57·3-15·3]
	HC	160	56·9 [11·6]	65·6		

Table S4. Sample demographics by modality. Abbreviations: aDS: asymptomatic Down syndrome group; dDS: Alzheimer disease dementia in Down syndrome group; IQR: interquartilic range; pDS: prodromal Alzheimer disease in Down syndrome group.

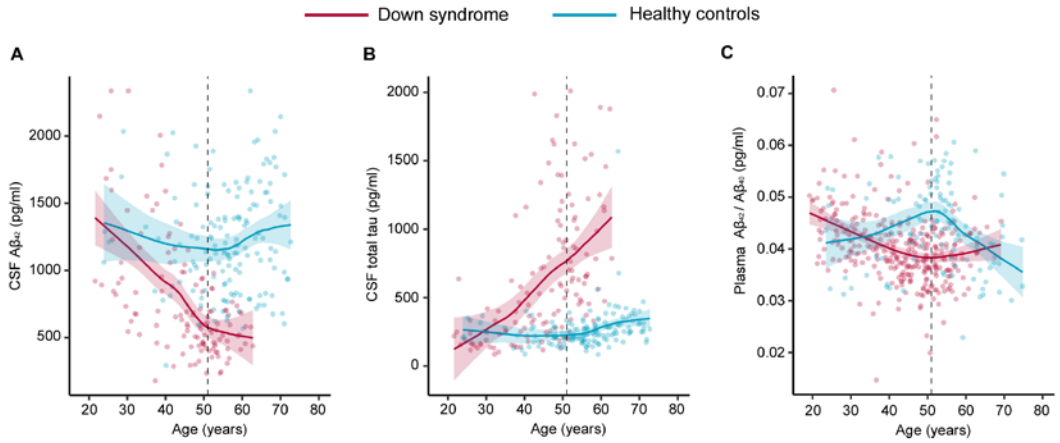

Figure S4. Biomarker changes with age in Down syndrome and controls. For each biomarker, individuals with Down syndrome are shown in red and healthy controls in blue. Shading represents 95% confidence interval. A. CSF Aß1-42 levels. B. CSF total tau levels. C. Plasma Aß40/42 levels.

The U-shaped association of plasma Aß1-42 with age might help reconcile conflicting evidence in the literature (Figure S4). Aβ1-40 and Aβ1-42 are the most extensively studied plasma biomarkers in Down syndrome. Previous studies have found increases in plasma Aβ1-42 and Aβ1-40 levels compared to euploid controls.[10–16] Most,[10,13,14] but not all[15,16] studies have found increases in Aβ1-40 or Aβ1-42 levels in individuals with DS and dementia, and the levels of Aβ1-40 and Aβ1-42 have also shown the capacity to predict future cognitive decline.[13,17] However, a decrease in Aβ1-42 levels and Aβ1-40 prior to cognitive decline has also been described.[18,19] These inconsistencies may be related to the timing of Aβ measures in relation to the development of symptomatic Alzheimer disease, given the dynamic nature of the underlying neuropathology.[18] In our work, plasma Aβ1-40 and Aβ1-42 were elevated in participants with Down syndrome, with differences between both biomarkers. The changes in plasma levels of Aβ1-40 were greater than those of Aβ1-42. This overproduction in Aβ1-40 relative to Aβ1-42 has previously been described.[12] Moreover, plasma Aβ1-40 levels increased with age in asymptomatic Down syndrome individuals, and were further elevated in those with prodromal Alzheimer´s disease and Alzheimer´s disease dementia. The pattern of change in Aβ1-42 was more complex. We found a non-linear trajectory of changes for Aβ1-42 levels. Plasma Aβ1-42 levels decreased with age until symptom onset, but were increased in the symptomatic stages of the disease. Plasma Aβ1-40 and Aβ1-42 changes are thus sensitive to disease progression. However, the weak correlation of Aß between the central and peripheral compartments,[8] together with the complex dynamics (where overproduction may be combined with decreased clearance)[20] make the interpretation of plasma Aβ measures in Down syndrome difficult. Further studies should assess the performance of the recently developed techniques using mass spectrometry which have demonstrated good accuracy to detect cerebral deposition.[9,21]

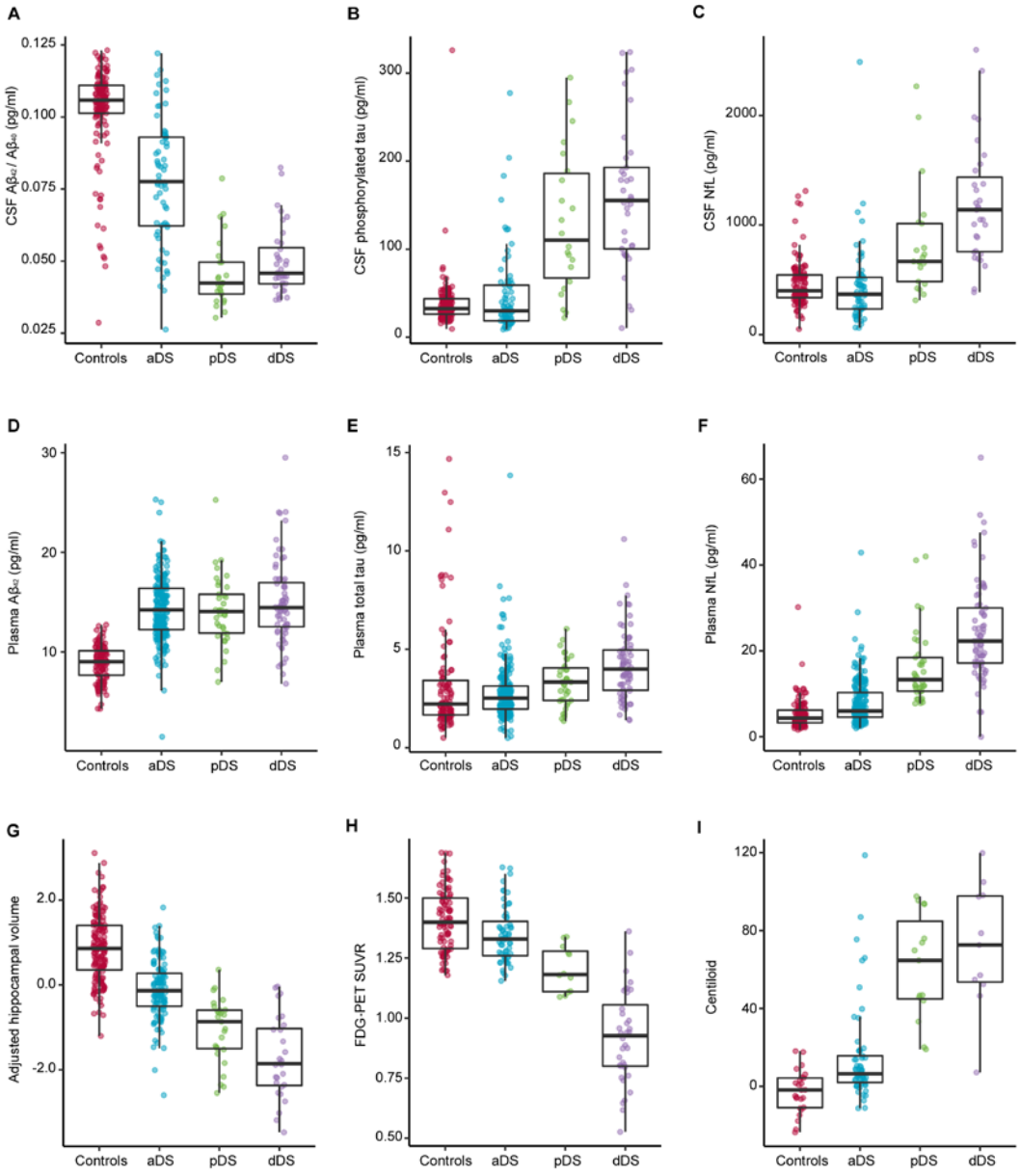

Figure s5. Biomarker changes with age in Down syndrome and controls, by group. A. CSF Aß1-42/1-40 levels. B. CSF p-tau levels. C. CSF NfL levels. D. Plasma Aß1-42 levels. E. Plasma t-tau levels. F. Plasma NfL levels. G. Adjusted hippocampal volumes. H. 18F- FDG PET in the Landau FDG signature. I. Centiloid levels. General population healthy controls, aDS= asymptomatic Down syndrome, pDS=prodromal Down syndrome, dDS=Alzheimer's disease dementia Down syndrome.

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